MYCORRHIZAE: Their Role in Tree Nutrition and Timber Production

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Introduction

In the course of history, men of science often discussed the roots of plants in terms of metaphors. The ancients compared roots with the mouth of plants. Liebig suggested that roots search for nutrients "as though they had eyes. . . " Darwin implied that "if the plant had a brain, it would lie in its roots." But the most objective brief statement, describing the essential feature of roots, was made by a man who was not a biologist and who spoke about an entirely different subject — Winston Churchill. The root, at this state of our knowledge, is "a riddle inside an enigma wrapped in a mystery." Churchill's formula is particularly applicable to roots of trees and other lignophytes which in addition to the mystery are usually wrapped in fungal mycelia.

The role of this mycelial addition to the root can be demonstrated by a very simple trial. If seed of most any tree is planted in a prairie or other grassland soil free from symbiotic fungi, the growth of plants will be arrested in the stage of the first whorl of leaves; the seedlings will exhibit signs of malnutrition and eventually die. This happens even in soils whose analyses show to contain an abundant supply of available nutrients. However, if prior to planting seed, the prairie soil receives about 0.1% by volume of a forest soil, the results will be radically different. Within a few weeks the surface and often the cortical cells of roots will be invaded by fungal mycelia and converted into root-fungus organs, or "mycorrhizae" of ectotrophic, endotrophic, ectendotrophic or peritrophic morphology. Simultaneously with the development of mycorrhizae the seedlings begin their normal growth (Fig. 1). This trial provides one of the most dramatic manifestations of the role which microorganisms play in the life of higher plants.

In the symbiotic relationships, the fungal partners not merely enhance, but enable the growth of plants. Photosynthesis is said to be the most important biochemical process on earth (Rabinowitch, 1945). For perhaps the largest part of the world's vegetative cover, photosynthesis is virtually dependent upon the presence in the soil of symbiotic microorganisms. This is particularly true of trees and other

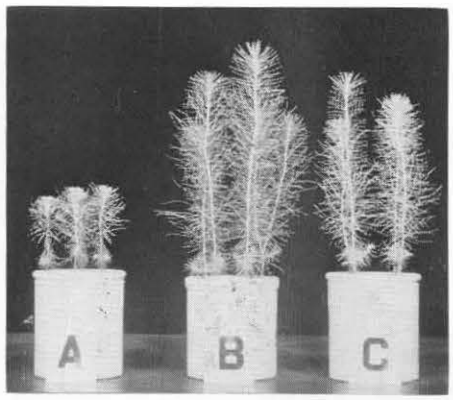

Figure 1. Effect of inoculation of a prairie soil with mycorrhiza-forming fungi on the growth of 6-month-old seedlings of Monterey pine, Pinus radiata*: A. Carrington prairie silt loam; B. The same soil with addition of 0.2% of Plainfield sand, a forest soil harboring mycorrhiza-forming fungus* Cenococcum graniforme*; C. Plainfield sand.*

lignophytes that render to us so many benefits in the form of wood, fruits, rubber, oleoresins, drugs, spices, and other commodities.

In spite of the enormous importance of mycotrophy, information on this mode of plant nutrition abounds in unsupported inferences (Imshenetskii, 1967). From its very beginning, the study of mycorrhizae was punctuated by speculative and contradictory claims.Mycorrhiza-forming fungi were portrayed as symbiotic components essential for the very existence of higher plants, as parasitic organisms harmful to their hosts, or as mere hitchhiking commensals of no particular importance to their vascular companions. Some research workers asserted the specificity of mycorrhizal associations, high site requirements of the lower symbionts, and their destruction by unfavorable environmental conditions or soil cultivation. Other investigators maintain that all forest soils, even those of cutover areas, harbor mycorrhiza-formers capable of symbiosis with nearly all tree species of the world. The most conspicuous illustration of the deficiencies in the knowledge of mycotrophy is provided by the minute space devoted to this phenomenon in the textbooks on microbiology and plant physiology. Many such textbooks do not even mention the term "mycorrhiza."

The principal cause of the inadequate progress in the study of mycotrophy must be sought in the complexity of the problem. Research dealing with mycorrhiza requires knowledge of mycology, plant physiology, biochemistry, soils, and plant nutrition, as well as some contact with either forestry or horticulture. As few specialists are capable of embracing all these fields, investigations are likely to be one-sided and semi-amateurish, rendering a large volume of unreliable information. Few other problems besides mycotrophy are in a greater need of team research.

This essay reviews the problem of mycotrophy in the light of studies conducted during the past quarter century by Wisconsin research workers (White, 1941; Rosendahl, 1942, 1943, Lafond, 1951; Mikola, 1952; Voigt, 1953; Wilde, 1954; Persidsky, 1955, 1960; Mader, 1956; Wallis, 1957; Matsui, 1958; Yatazawa, 1960; DeVries, 1962; Iyer, 1964, 1965; Spyridakis, 1965; and Lipas, 1968). The author gratefully acknowledges helpful suggestions rendered by his colleagues, Professors Gordon Chesters, R. B. Harhis, T. T. Kozlowski, J. E. Kuntz, and R. J. Muckenhirn.

The first part of the treatise deals with the three riddles of the mycorrhizal relationship.

Unsolved Problems of Mycorrhizae

ABSENCE OF TREE SYMBIONTS IN PRAIRIE AND OTHER GRASSLAND SOILS

Experience in different parts of the world has repeatedly demonstrated that many grassland soils do not harbor fungi symbiotizing with trees. As suggested by our studies, this biological idiosyncrasy is largely responsible for the persistence of the intra-zonal prairies in the humid climate of the American Midwest (White, 1941).

In prairie soils of Wisconsin supporting windbreaks, the distribution of mycorrhiza-formers is strictly delineated by the extension of tree roots (Mikola, 1953).

During the past 60 years, the absence of mycorrhiza-formers in grassland soils has caused numerous failures of nursery stock and afforestation seedings in Russia (Wissotsky, 1902), India (Narashimhan, 1918), Australia (Kessell and Stoate, 1938), Rhodesia (Kelley, 1950), United States (McComb, 1938), and other countries. In all these cases, the remedy was found in the simultaneous introduction into grassland soils of the 2 symbionts: the fungus and the tree.

One of the plausible explanations of the absence of tree symbionts in grasslands is that the roots of herbaceous vegetation excrete toxic substances which preclude the survival of mycorrhizal fungi in the absence of host plants (Curtis and Cottam, 1950; Persidsky *et al.*, 1965).

UNAVAILABILITY OF NUTRIENTS OF PRAIRIE SOILS TO TREES IN THE ABSENCE OF SYMBIOTIC FUNGI

Most puzzling is the fact that non-mycorrhizal tree seedlings are unable to utilize nutrients of chernozems and other soils of grasslands. Not only young plants, originated by direct seeding, but vigorous non-mycorrhizal seedlings, produced in hydroponics, perish from starvation in prairie soils which according to soil analyses have a plentiful supply of available nutrients and which may produce as much as 150 bushels of corn per acre (White, 1941; Rosendahl, 1943). Usual explanations of this discrepancy include inherent inefficiency of tree roots in extracting colloid-adsorbed ions and a severe competition of microorganisms for the readily available fraction of nutrients. Our knowledge at this time covers only two details that have bearing on this problem.

Trees raised in sand cultures or solutions exhibit a very high nutrient utilization capacity (Olson, 1944; Wilde and Spyridakis, 1967); in such media they attain a double or a triple rate of growth in comparison with fertile soils.

Treatment of prairie soils with allyl alcohol has caused a replacement of the native microflora by *Trichoderma viride*, a saprophytic fungus, and induced an elephantine growth of the crowns of tree seedlings (Fig. 2). Among other changes, application of allyl alcohol considerably increased the supply of available phosphorus (Wilde *et al.*, 1956; Yatazawa *et al.*, 1960). According to some observations, addition of phosphate fertilizers returned the mycorrhizae-free prairie soils to the productive state for tree seedlings (Hatch, 1937; Miller, 1938; McComb, 1943). This suggests that in some instances phosphorus plays a part of the "open sesame" for tree nutrients locked up in mycorrhiza-free soils. Unfortunately, the magic command, given in the form of fertilizer applications, does not work in many cases (White, 1941; Shemakhanova, 1962).

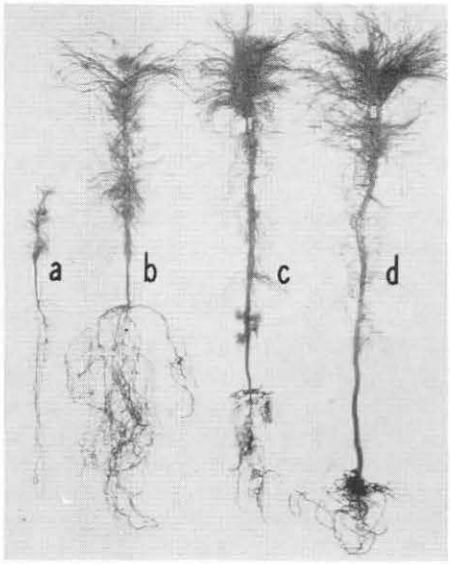

Figure 2. Effect of allyl alcohol on the growth of 1-year-old Monterey pine, Pinus radiata, *in virgin soil. a. Control; Carrington silt loam; b. Soil inoculated with symbiotic fungi by an addition of 0.2% by volume of a forest soil; c. Soil treated with 100 gallons per acre of allyl alcohol; d. Soil treated with 200 gallons per acre of the same chemical. Note the difference in the development of root systems and the absence of mycorrhizal short roots in all plants (After Yatazawa* et al., *1960).*

SURVIVAL OF MYCORRHIZAL FUNGI IN DEFORESTED SOILS

Observations of R o s e n d a h l and Wilde (1942) revealed that mycorrhizal fungi survive in soils of old cutover lands, including those which had been subjected to cultivation or grazing for as long as 40 years. These findings were questioned by the Swedish students of mycotrophy. A repeated study, however, disclosed the presence of mycorrhiza-formers in soils of Sweden which were under agricultural use for a period exceeding 100 years (E. Björkman, personal communication).

It is possible to theorize about either anabiosis of mycorrhizal fungi in the absence of suitable host plants, their saprophytic mode of existence, or their subdued symbiosis with herbaceous vegetation. Be that as it may, the persistence of tree symbionts led Wilde (1954) to consider their presence as the positive identifying feature of forest soils and to declare: "Once forest soil, forever forest soil."

Facts and Fallacies

There is uncertainty about many other aspects of the symbiotic relationship, such as identity of mycorrhiza-formers, importance of mycorrhizal short roots, mechanism of the nutrient uptake, survival of symbionts in the presence of biocides, and influences leading to development of ectendotrophic mycorrhizae and "pseudomycorrhizae." The roles played by mycelia dwelling inside cortical tissues of roots and those comprising a part of the rhizosphere are especially in need of elucidation. The following discussion touches upon the most salient misbeliefs of the mycotrophic literature.

SHORT ROOTS AND LONG MYCELIA

The symbiosis of fungi and higher plants was originally classified into two distinct forms, termed ectotrophic and endotrophic mycorrhizae (F r a n k, 1885). The ectotrophic mycorrhiza develops club-like "short roots" with a

thick mantle and intercellular mycelia forming in the cortex so-called "Hartig net." The endotrophic mycorrhiza lacks short roots, but exhibits within the rootlets intracellular hyphae, termed vesicles and arbuscules.

Students of tree mycotrophy have given most attention to the ectotrophic mycorrhizae, particularly to the structure and function of short roots. Many investigators considered these appendages vital to nutrition. Consequently, a great deal of time was spent in counting short roots and speculating about their role in the uptake of nutrients.

Most likely these misguided efforts were induced by observations of young seedlings of greenhouse cultures or nursery beds, i.e., plants exposed to full light and manufacturing excess of carbohydrates. In trees reproduced under the canopy of mother stand, short roots are either entirely absent or very few in number and their absorbing surface is insignificant.

Fassi (1967) studied mycorrhizae of autochthonous red and white pine in prairie-forest and podzol regions of Wisconsin. He reported a vast difference in the abundance of ectotrophic short roots of seedlings produced under artificial conditions of forest nurseries and those of indigenous trees (Figure 3). Scarcity of mycotrophic organs was particularly common to excavated deep roots of 15- to 30-year-old pines (Figure 4). These observations verified the 80-year-old statement of Hartig (1886) who found "not a trace of mycorrhizae" on roots of vigorously growing trees.

There is undeniable knowledge of a correlation between the adsorbing surface of root systems and the growth of plants (Wilde and Voigt, 1949; Carley and Watson, 1965), but a highly contradictory body of information on the growth influence of either density or structure of short roots (Harley, 1937). The very fact that forest stands include untold numbers of rapidly growing trees of endocellular and "autotrophic" morphology, i.e., trees entirely free from short roots, testifies to the subordinate importance of these appendages.

In large measure, the undue emphasis on short roots owes its origin to the failure of many investigators to extend their observations beyond the microscopic slide into the soil and the rhizosphere. In consequence, the minute hyphae of mycorrhizal rootlets obscured their view of the forest and its important attribute, the extra-matrical mycelia entwining the roots and an enormous volume of soil particles. According to Burges and Nicholas (1961), the length of fungal hyphae in the surface layers of some soils varies between 4 and 6 meters per millimeter of soil volume.

SYMBIOSIS: OBVIOUS AND CONCEALED

Frequent statements in the literature about the growth of non-mycorrhizal or "autotrophic" trees are in cardinal disagreement with observations in Wisconsin. Research in this state revealed that there are no forest soils without mycorrhizal fungi (White, 1941; Rosendahl, 1943; Rosendahl and Wilde, 1942). On the other hand, all our information indicates that there are no trees, naturally or artificially established, which produce normal growth without a direct beneficial influence of symbiotic fungi. This influence is effected not only by the ectotrophic or endotrophic fungal hyphae but also by peritrophic (peripheral) or juxtaposed mycelia producing no morphological or anatomical modification of roots. This type of *crypto-symbiosis* was demonstrated by planting in prairie soils the naturally reproduced tree seedlings of autotrophic morphology, as well as hydroponic-raised seedlings.

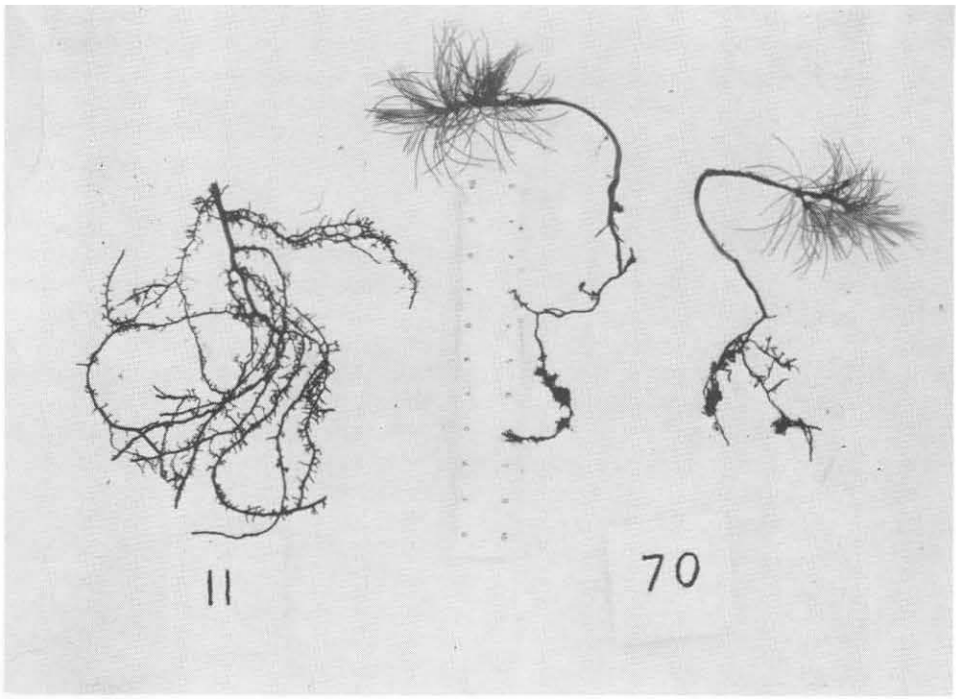

Figure 3. Representative roots of 3-year-old white pine, Pinus strobus, *of different origin: 11 - nursery seedlings from the Griffith State Nursery of Wisconsin with abundant ectocellular and ectendocellular short roots; 70 - seedlings naturally reproduced under the canopy of mother stand near Clear Lake, Wisconsin, with some mycoplasts, but very few ectocellular short roots (Italfoto, Torino).*

In some trials, hydroponic plants were placed in a forest soil for 2 weeks prior to their planting in a prairie soil (Iyer, 1964). The results were invariably the same: trees raised in sterile nutrient solutions failed to grow in prairie soils after they had produced the initial whorl of leaves; seedlings that were in contact with a forest soil grew vigorously, and *in some cases* eventually developed mycorrhizal short roots (Fig. 5).

These observations are in agreement with the following statement by Harley (1959): "Those associations between roots and microorganisms which have been given the name mycorrhiza appear to be only the end terms of numerous less specialized tendencies for roots and microorganisms to set up balanced relationships with one another."

ECTENDOTROPHIC MYCORRHIZAE AND PSEUDOMYCORRHIZAE

These varieties of mycorrhizal or pseudomycorrhizal short roots are of no greater importance to the growth of tree seedlings than are the normal short roots. Nevertheless, the claims of "mild" or "partial" parasitism, attached to these deviators from the common morpho-anatomical pattern of development, perturb all concerned: the student of mycotrophy, the nurseryman, and the plant pathologist.

The ectendotrophic mycorrhizae exhibit several structural irregularities, such as the absence of a mantle, over-

Figure 4. Jet-excavated root of a rapidly growing 16-year-old red pine exhibiting extremely small number of mycorrhizal short roots (Coloma loamy sand, Greenwood Refuge, W.C.D., Waushara County, Wisconsin).

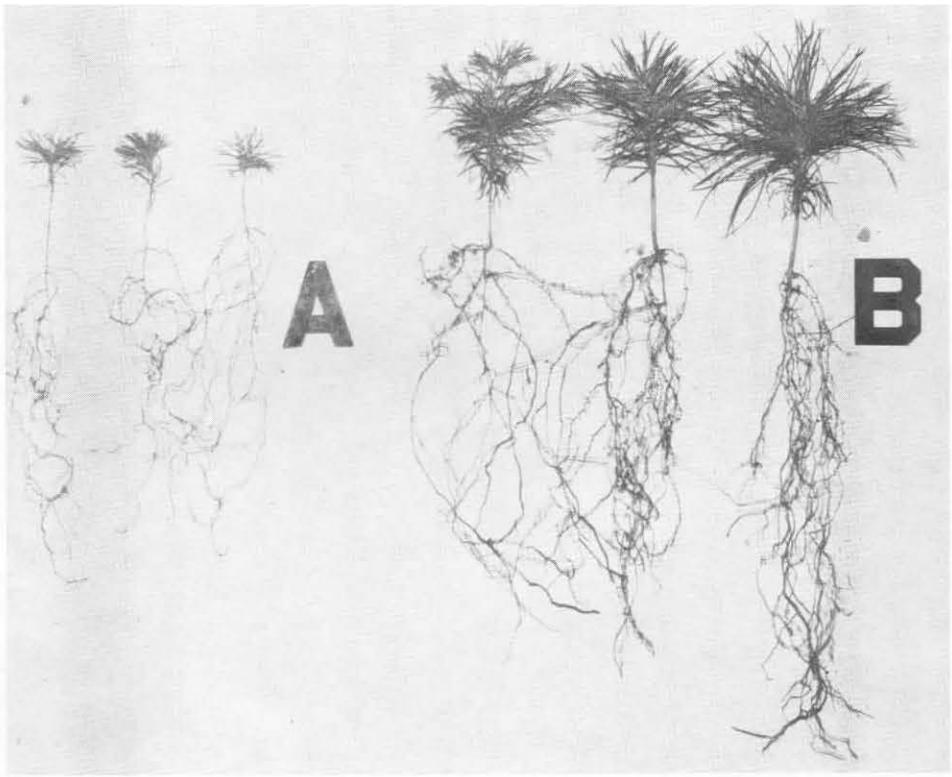

Figure 5. Average seedlings of 9-month-old white pine, Pinus strobus, *raised for 2 months in nutrient solutions and then transplanted to Parr silt loam prairie soil: A. Seedlings transplanted directly from hydroponics to prairie soil; B. Seedlings inoculated with mycorrhizal fungi by hilling hydroponic stock for 2 weeks in Plainfield forest sandy soil prior to their transplanting to prairie soil (After Iyer, 1964).*

development of the mantle and Hartig net, and the presence of both intercellular and intracellular mycelia (Möller, 1902; von Tubeuf, 1903). These transitional forms are now regarded either as beneficial "ectendotrophic mycorrhizae" (Björkman, 1942; Mikola, 1948), semi-parasitic developments (Melin, 1923), a harmful "haustorial infection" (Levisohn, 1963), or as a phenomenon which is too little known to be classified (Dominik, 1956). The research of Mikola (1965) and Laiho (1965), conducted in European and North American environments, has helped to reduce the scope of contradictions and cast new light on the nature of the ectendotrophism.

Typical ectendotrophic short roots are dichotomous, without or with a very thin mantle. The inside cells of the cortex are hypertrophied and exhibit both intercellular and intracellular mycelia (Fig. 6). One of the fungi producing ectendotrophic short roots was isolated by Mikola, but was not identified because of the absence of reproductive organs. It was tentatively designated as E-strain with addition of the number of isolate, e.g., E-57. Inoculation under aseptic conditions rendered ectendotrophic mycorrhizae with Scotch pine,

Pinus silvestris, but ectotrophic mycorrhizae with Norway spruce, *Picea Abies*.

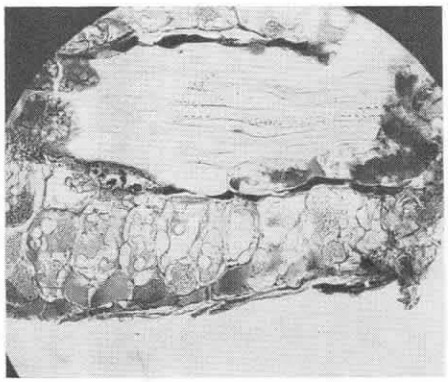

Figure 6. Cross-section of ectendotrophic short root of Pinus Silvestris *exhibiting hypertrophied cortex cells with both intercellular and intracellular mycelia (After Mikola, 1965).*

Both Bjorkman (1942) and Mikola (*l.c.*) observed ectendotrophic short roots largely on trees growing in soils of low fertility, such as those of heavily cropped forest nurseries, abandoned farms, and burned-over lands. Transplanting ectendotrophic seedlings to uncultivated and unburned forest soil resulted in rapid development of ectotrophic short roots. On abandoned fields, however, the replacement of ectendotrophic short roots was not accomplished until the next growing season.

Experiences in Wisconsin nurseries provide a few supplemental details pertinent to this amphimorphic type of mycorrhizae. The ectendocellular short roots are predominantly, perhaps entirely, confined to red pine, *Pinus resinosa*, jack pine, *P. banksiana*, and Scotch pine, *P. silvestris;* such roots are not formed, or formed in very rare instances, on white pine, *Pinus strobus*, white spruce, *Picea glauca*, and Norway spruce, *P. Abies*. Hence, the development of this type of short roots is common only to tree species which are particularly vulnerable to damping-off disease, caused by *Rhizoctonia solanum*, *Pythium de Baryanum* (Roth and Riker, 1943). and occasionally *Phytophthora* spp. (Fassi, 1966; personal communication).

Experiences in Wisconsin nurseries have indicated that the incidence of damping-off has greatly diminished when the addition of peat and leafmold has increased soil organic matter. A study of Mikola (1952), conducted under controlled conditions, substantiated the beneficial effect of humus. Hand in hand with the improvement of fertility of nursery soils, there was also a marked decrease in occurrence of ectendocellular mycorrhizae. In the middle 1950's the nurseries of Wisconsin produced predominantly seedlings with ectocellular short roots even on soils treated with biocides (Wilde and Persidsky, 1956). These observations suggest that damping-off and ectendocellular fungi have a similar distributional amplitude with reference to both soil conditions and host plants. On the other hand, no evidence was obtained to substantiate the claims of Delacroix (1897), implying a possibility of a transition from symbiosis to parasitism and vice-versa.

As regards the "mild" or "partial" parasitism, caused by whatever fungi, the least said, the better. Parasitism of this kind simply does not exist in forest nurseries, at least not in Wisconsin nurseries. A deterioration of nursery stock by some unidentified parasite could not have passed unnoticed by nurserymen and a score of research workers connected with the annual production of some 40 million trees.

From the standpoint of nursery practice, the occurrence of thin-mantled short roots with intracellular hyphae has one practical implication; it usually

indicates a depletion of soil fertility, danger of damping-off, and need for addition of organic remains and adjustment of the nutrient balance.

One of the badly confused concepts in the realm of mycotrophy is "pseudomycorrhiza." Melin (1923), who introduced this nomen, believed that short roots of this type are formed by non-mycorrhizal fungi, such as *Mycelium radicis atrovirens*, and represent a mild form of parasitism. At a later date, suggestions were made to identify pseudomycorrhiza with ectendotrophic mycorrhiza, or to eliminate the term pseudomycorrhiza as superfluous. Yet, regardless of its semantic inadequacy, this term may have a suitable niche in the pertinent vocabulary.

Some tree species, particularly pines, have an inherent tendency to produce branched or forked short roots even when they are raised in sterile nutrient solutions (Fig. 7). Contamination of such solutions with common molds usually increases the number of these organs, probably under the influence of growth promoting substances excreted by fungi. As revealed by prairie soil tests, these "virgin short roots" (in Persidsky's terminology) have no relation to mycotrophy; they justly could be named pseudomycorrhizae. The development of these appendages of uncertain physiological function received a great deal of attention in work of Slankis (1949, 1958).

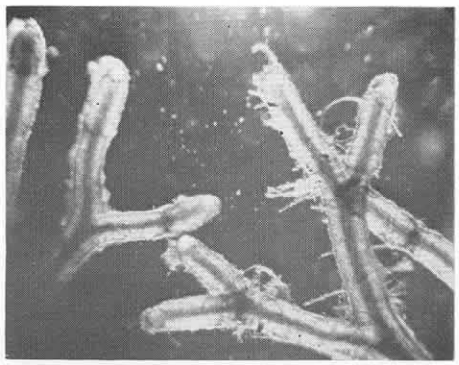

Figure 7. Forked short roots of red pine, Pinus resinosa, *without intercellular or intracellular hyphae; an example of a "pseudomycorrhiza" developed in a nutrient solution free from mycorrhiza-forming fungi.*

SPECIFICITY OF MYCORRHIZAL SYMBIONTS

The idea that trees form symbiotic unions only with certain species of fungi very likely was suggested by common names of mushrooms. For example, the Russian names of aspen, birch, pine, and oak mushrooms refer to different *Boletineae*. The suggested relationship, however, has an untold number of exceptions; in spruce forests of Ontario, the most abundant is *Boletus rufus*, the "aspen mushroom;" the "birch mushroom," *Boletus scaber*, is just as common in aspen as in birch stands of Wisconsin. Many more examples might be cited.

The extremely wide adaptation of trees to form a symbiotic union was demonstrated by one of Rosendahl's trials. Trees indigenous to distant parts of the world (U.S.A., Mexico, Bohemia, Ural Mountains, and Japan) were raised from seed in Wisconsin prairie soils and also in those inoculated by an addition of a small amount of forest soils; the latter included dune sand, lacustrine clays, sandy podzol (A_2 horizon), and gley loam. Without exception, trees did not grow beyond the first whorl of leaves on symbiont-free prairie soils, but developed normally on such soils mixed with 0.1 or 0.2% of any forest soil. Later, Wilde successfully inoculated Wisconsin prairie soils with forest soils of distant lands and extremely unfavorable composition, e.g., near-permafrost layers of Alaska, ferralized clays of India, and greywacke loams of New Zealand (Fig. 8).

Thus, the symbiotic relationship of lignophytes is foreign to segregation: a tree of any descent finds a suitable partner in any soil harboring mycorrhizal fungi, and several thousand miles distance between birthplaces of the 2 symbionts presents no obstacle to their union.

The results achieved with efficient strains of nitrogen-fixing bacteria (Fred *et al.*, 1932) gave rise to the idea that inoculation of tree seedlings with selected species of mycorrhizal fungi would lead to increased growth of plantations. This assumption, however, is not entirely sound; nodule bacteria enrich soil in an essential element, whereas mycorrhizal fungi only increase the solubility of nutrient substances present in the soil. Contrary to some claims (Moser, 1956), in no Wisconsin trials did the origin of inoculum ever produce a detectable deviation in the growth of tree seedlings; growth was invariably correlated with soil fertility. These experiences suggested that mycorrhizal fungi act largely as quasi-catalytic agents.

One of the most serious obstacles to introduction of especially efficient "plus" symbionts into soils of nurseries or planting sites is the antagonistic behavior of indigenous mycorrhizal fungi (Mikola, 1965; Laiho, 1965).

On the other hand, assumptions that *any* fungus can form mycorrhizae (McArdle, 1932; Kelley, 1950) are utterly without foundation. Prairie soils harbor a multitude of fungi, none of which form mycorrhizae with trees. Introduction of *Mucor, Aspergillus*, and *Alternaria* spp. into sterilized soils increased soil acidity and availability of phosphorus, but failed to produce mycorrhizae with pine seedlings, as revealed by their root morphology and subsequent failure to grow in prairie soils (Rosendahl, 1943).

In a very general way, there seems to be broad differentiation among the mycotrophic organisms, as judged by the structure of mycorrhizae: soils of heavily cropped nurseries and depleted farm lands usually produce seedlings with ectendocellular mycorrhizae; podzolized, raw humus soils are characterized by predominance of trees with ectocellular mycorrhizae; non-podzolic soils with mull humus and cryptorganic soils often support trees with peripheral mycorrhizae; the occurrence of endocellular mycorrhizae is rather sharply delineated by the taxonomy of lignophytes.

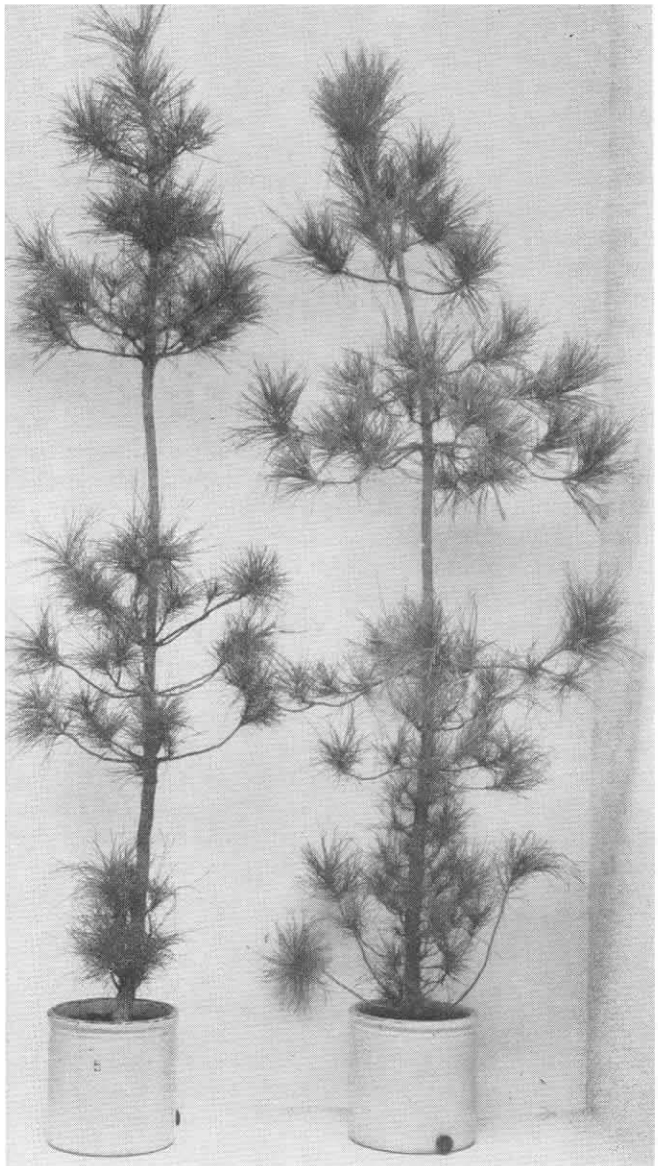

Figure 8. Four-year-old Monterey pine, Pinus radiata, *raised from seed in Carrington prairie silt loam with addition of 2 g per jar of soil from indigenous stand of* Nothofagus *at Taita Experimental Station, New Zealand.*

Mechanism of Mycotrophy

Difficulties in understanding the mechanism of mycotrophy begin with the existing outmoded terminology.

The terms ectotrophic, endotrophic, and ectendotrophic mycorrhizae are ambiguous and objectionable for several reasons. In literal translation these expressions mean "outside-" and "inside-feeding fungal roots." Who is feeding whom and what is a moot question. Moreover, nutrition is a physiological function and has no place in the realm of plant morphology and plant anatomy. In consequence, Wilde *et al.* (1967) suggested a replacement of these terms by expressions: *ectocellular*, *endocellular*, and *ectendocellular mycorrhizae*.

Regardless of terminology, however, these morpho-anatomical varieties are of highly questionable importance for nutrition of either the tree or the fungus. The roots with internal hyphae are far less frequent in stand-forming trees than roots with superficial or peritrophic mycelia.

The peritrophic mycorrhiza was described by Jahn (1934) as a superficial mantle whose hyphae do not enter root tissues. This type is common in seedlings of both hardwoods and conifers, and is encountered particularly often in soils with reaction exceeding pH 6.0. Most likely the entrance of fungal hyphae in such soils is precluded by a more rapid development of a strong epidermis of rootlets. In trees endowed with nitrogen-fixing mechanisms, such as *Robinia*, *Alnus*, and many trees of the tropics, mycorrhizal association is usually in the form of peripheral mantles of mycelia or mycelia-penetrated organo-mineral mash.

The detection of extra-matrical mycelia requires a very careful handling of the root system; the network of hyphae may be easily overlooked or detached in the process of tree lifting. This explains why the concentration of fungus mycelia around the roots of trees, described by Theophrastus 17 centuries ago, only recently received attention of research workers.

One of the diligent students of the morphology of mycorrhizae, Dominik (1957), made the following observations on the superficial contact of fungus mycelia: 'Species lacking their natural fungal symbionts and seemingly autotrophic are invariably surrounded with abundant hyphae of *Cenococcum graniforme*. The capacity of the latter for symbiosis with various plants is widely known. Dense agglomerations of these hyphae often merit the name peritrophic 'mycorrhiza'." Fungal mantles with no distinct connection with root tissues were reported

by Prat (1926), Lobanow (1960), Fassi (1960), Pachlewski and Gagalska (1964), and Laiho (1965).

A close relative of peritrophic mycorrhiza is "superficial mycorrhiza," which has a firmer contact with the root tissues (Clowes, 1951). The 2 forms, however, are usually present on the same root system. Their subdivision is likely to be confined to printed pages.

Notwithstanding their omnipresence and paramount ecological importance, the epirhizal mycelia thus far failed to acquire suitable terminology.

The expression "peritrophic mycorrhiza" is derived from the Greek *peri* for "around" or "about," and *trophe* for "nutrition." This is a rather ambiguous term, and if this form of a rhizospheric association deserves to be classified as "mycorrhiza," it should be *peripheral mycorrhiza*. Actually, the superficial mycelia hardly form a "root-fungus organ," and a term *rhizoclena*, from the Greek *rhiza* for root and *clena* (or *chlena*) for mantle, appears to be more appropriate (Wilde and Lafond, 1967).

In a detailed classification, rhizoclenae may be subdivided into 2 principal varieties (Fig. 9):

mycochlamydes, or lanose fungal mantles covering roots circumferentially or partially (term from the Greek "chlamys" for cloak or mantle);*

mycoplasts, or grumose fungal mantles consisting of clovate sapropellous clusters of soil, root sloughings, and fungal hyphae, agglutinated around the terminal parts of roots by humate suspensions (term from the Greek "plastus" for granule).

The fimbrial root auxiliaries are important constituents of the rhizosphere with its chelating processes that convert raw organic and mineral sources of nutrients into a form available for utilization by either mycorrhizal or non-mycorrhizal roots of trees (Spyridakis, 1965). It is not entirely metaphoric to state that a tree removed

*Excellent illustrations of mycochlamides on roots of *Pinus ponderosa* are given by Goss (1960, Figs. 1 and 2).

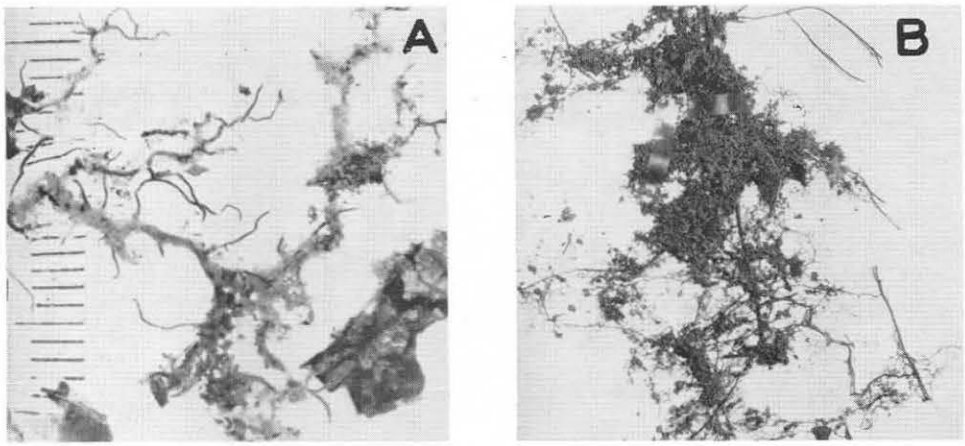

Figure 9. Different forms of extra-matrical mycelia: A. Mycochlamydes, *fungal mantles on roots of* Gilbertiodendron dewevrei *De Wild in equatorial forest of Congo (After Fassi, 1960); B.* Mycoplasts, *grumose, mycelia-agglomerated organo-mineral clusters on roots of* Pinus strobus *L. in northern Wisconsin. These fimbrial root auxiliaries play an important part in the production of chelating agents and conversion of raw sources of nutrients into available form.*

from the soil is only a part of the whole plant, a part surgically separated from its rhizospheric digestive organ. In a dictionary definition, the rhizosphere is the part of soil influenced by the roots of plants. The influence of the rhizosphere on roots and, in turn, on the growth of plants is of no lesser importance!

Results of studies conducted with Wisconsin soils are in agreement with the mineral theory of Stahl (1900); the ability of mycorrhizal fungi to release available nutrients from prairie soils, peat soils, and humus-free geological matrix was repeatedly demonstrated (White, 1941; Rosendahl, 1942, 1943; Wilde, 1946; Stone, 1950; Wilde and Iyer, 1962). Table 1 includes particularly informative results obtained by Rosendahl (1942) with pure culture of *Boletus felleus*.

On the other hand, results of Wisconsin studies have shown much lesser agreement with ideas pertinent to the role of endorhizal or root-ingrown mycelia in actual nutrition of the host plant, i.e., in the manufacture of nutrients available to trees and the uptake of nutrients via fungal mycelia.

The possibility of a transfer of nutrients through mycelial mantles was first suggested by Kamienski (1884), who stated: "There is no way by which nutrient solutions can pass and reach the roots of *Monotropa*, except through the mycelium." More recently, the ability of fungal mycelia to transport nutrients from a solution to the roots of host plants and vice-versa was demonstrated by the use of isotopes (Melin and Nilsson, 1950, 1952, 1957; Mosse, 1959). The capacity to transmit nutrient ions from a solution is, of course, inherent to many tissues, aside from fungal mycelia. Under laboratory conditions, cotton and nylon strings transmitted isotopes by capillary action from a solution to the roots and the foliage of hydroponic raised seedlings (Iyer, 1964). As shown by our trials, employing P^{32} and S^{35}, not only non-mycorrhizal roots, but even moss-covered branches of black spruce before layering, absorbed and transmitted nutrients from solutions to the foliage with a rapidity far exceeding that of mycelial transfer. As observed by Kuntz and Riker (1956), it took only 20 minutes for sodium Iodide-131 and Rubidium-86 to be transported upward from roots to the foliage, a distance of 35 feet.

A transfer of dissolved nutrient ions, however, has little to do with the actual nutrition of trees which is largely dependent upon the solid phase feeding, *i.e.*, extraction of available nutrients

TABLE 1. Dry weights and potassium contents of average red pine seedlings grown in sub-irrigated quartz sand-orthoclase cultures for 6 months and subsequently in Carrington prairie silt loam for 16 months (After R. O. Rosendahl, 1942).

Inocula	Dry weight of seedlings g	Content of K p.ct.	Content of K mgs
6-month-old seedlings grown in sand-orthoclase cultures			
Control	0.286	0.35	1.00
Amanita muscaria	0.364	0.34	1.24
Boletus granulatus	0.302	0.31	0.94
Boletus felleus	0.935	0.53	4.95
22-month-old seedlings grown in prairie soils			
Control	0.567	0.54	3.10
Amanita muscaria	0.457	0.56	2.52
Boletus granulatus	0.583	0.58	3.38
Boletus felleus	2.325	0.97	22.55

from raw organic matter and unweathered minerals. This transformation of nutrient ions from an inert to a utilizable state can be accomplished only through close contact of soil particles with living matter, predominantly with fungal mycelia which form a very large soil-tissue interface.

The maximum concentration of fungal hyphae is in the immediate proximity of roots, or in the rhizosphere. The rhizosphere is a medium enriched in root excretions, discharges of organisms, products of decay of both roots and microbes, and chelating compounds, i.e., weak organic acids of EDTA type that possess complexing properties. The effect of chelation together with activity of microorganisms is largely responsible for disintegration of raw soil materials and release of available nutrients (Leaf, 1957; Spyridakis *et al.*, 1967; Pochon and de Barjac, 1956; Müller, 1965). Table 2 includes a few results from the extensive study of Spyridakis (1965), illustrating a transformation of biotite mica under the influence of root systems and root sloughings of coniferous seedlings.

In forest soils an essential part of the rhizosphere consists of epirhizal mycelial mantles. These generally overlooked peripheral auxiliaries of root systems serve as centers of chelating processes that manufacture available nutrients (Fig. 9). Table 3 shows the difference between the contents of available nutrients in the soil adhering to root systems and the soil of interrhizal spaces (Iyer, 1964). The chelating effect of the rhizosphere attains particular importance in soils enriched in feldspars, micas, and other silicate materials, and influenced by capillary fringe present at a depth from about 4 to 9 feet (Wilde and Iyer, 1963; Wilde *et al.*, 1965).

The beneficial effects of fungi on higher plants may be expressed in several forms; all of these, however, present segments of the same general phenomenon: *rhizospheric symbiosis* (Wilde, 1958).

A fraction of available nutrients, released in the rhizosphere, is undoubtedly utilized *in situ*, being absorbed by either mycorrhizal or nonmycorrhizal roots. Another fraction is transported in the form of humate suspensions by gravitational discharge; it may enrich subterranean water, become a part of the accumulative horizon, or be intercepted by the deeper part of tree root system.

Recent studies have considerably broadened the theory of nutrient uptake. The introduced "carrier" concept of ionic migration (Overstreet, 1957; Jackson and Stief, 1965; Rauser and Hanson, 1906) has a direct bearing on the old problem of the inability of grassland soils to nourish trees.

TABLE 2. Weathering of biotite mica under the rhizospheric influence of 1-year-old tree seedlings and the effect of electron beam-sterilized and unsterilized root sloughings on release of water- and *N* NH_4OAc — soluble potassium after 9-month incubation (After D. E. Spyridakis, 1965).

Tree species	Biotite size particles under 2u p.ct.	Biotite contents of Al_2O_3 p.ct.	Release of K with sterile root sloughings mg.	Release of K with unsterilized root sloughings mg.
Control, unweathered biotite	0.6	11.8	2.63	2.63
Jack pine	1.1	14.2	3.98	14.67
White pine	8.3	21.5	5.69	20.60
Hemlock	10.6	21.7	5.97	15.82
White cedar	12.4	22.5	5.14	17.67

TABLE 3. Average concentration of major nutrient elements in soils adherent to roots of 5-year-old indigenous seedlings of red and white pines and soils collected in interrhizal spaces (After Iyer, 1964).

Origin of Soil Samples	Reaction pH	Exch. capacity m.e./100 g.	Avail. N	Avail. P	Avail. K	Exch. Ca	Exch. Mg
			p.p.m.			m.e./100 g.	
Interrhizal spaces of coarse outwash sand	4.6	2.1	Tr.	12	29	0.55	0.22
The same soil adherent to roots of *Pinus resinosa*	4.5	3.7	24	64	78	1.65	0.61
Interrhizal spaces of podzolic sandy loam	4.7	2.8	16	42	31	1.00	0.32
Aggregates of the same soil around roots of *Pinus strobus*	5.0	4.1	92	80	115	2.44	0.72

The restricted diffusion of nutrient ions in and out of roots of healthy plants suggested participation of cellular constituents in the penetration of nutrients through cell membranes and in bonding of nutrient ions in an energized state. The movement of different nutrient ions into and within plant tissues is said to be accomplished by separate mechanisms requiring the presence of specific cellular constituents and protoplasmic sites (Bange and Hooymans, 1967). On the other hand, transport of nutrient ions from dilute solutions into roots requires energy derived from aerobic respiration. The principal source of this energy is provided by compounds of covalent bonds, possibly lipoproteins, that are integral parts of cell membranes (Hanson, 1967).

The endocortical and epirhizal mycelia are intimately connected with root tissues. As shown by the use of isotopes, they are efficient transmitters of soil solution and are inseparable components of the nutritional mechanism of symbiotrophic plants. In turn, the fungal accessories of roots cannot be without influence on energy relations, the nature of cellular constituents, and other factors determining the fate of nutrient ions.

Analyses of both nursery stock and seedlings raised in the greenhouse suggest that microbiotic influence is especially pronounced in the case of phosphorus whose uptake by woody plants virtually requires some constituents provided by the fungus. Studies of phosphorus mycotrophy, including comprehensive investigations of Harley and his collaborators, were well summarized by Shemakhanova (1962). The unavailability of phosphorus was blamed in the past for the refusal of prairie soils to provide an adequate nutrition to forest trees (Hatch, 1937; Miller, 1938; McComb, 1943). More recently, Daft and Nicolson (1966) observed a particularly conspicuous participation of fungal symbionts in the uptake of phosphorus. Similar evidence was recorded in our treatments of prairie soils with allyl alcohol. (Fig. 2.) This chemical induces a replacement of native microflora with *Trichoderma viride*, a fungus much more efficient in conversion of organic phosphorus into available form than the native microflora (Yatazawa *et al.*, 1960). Recently, Henderson and Stone (1967) and Lipas (1968) observed that a suppression of mycorrhizal fungi in nursery soils by fumigants precludes the uptake of phosphorus by tree seedlings, even when this nutrient element is present

in the form of easily soluble or "available" compounds.

The fungal associates of tree roots are not necessarily the only participants in symbiotrophy of forest stands. On areas supporting *Vaccinium* spp., *Epigaea repens*, *Gaultheria procumbens*, and other heath plants, roots of trees are in close contact with those of the ground vegetation. The ericoid and arbutoid mycorrhizae of the latter are usually accompanied by varieties of *Mycelium radicis*, the black *Cenococcum graniforme*, and other soil organisms. These constituents of the rhizosphere may also augment the nutrient supply of trees by effecting the breakdown of raw humus and silicate minerals.

The tremendous capacity of tree roots to extract nutrients from unweathered minerals was disclosed in a study of red pine, *Pinus resinosa*, plantations established on humus-free gravelly substrata, exposed by a removal of a 2-foot layer of the surface soil (Wilde and Iyer, 1962). These plantations have attained a site index of 57, and within 32 years their leaf fall has enriched the surface 6-inch layer of the geological matrix in available major and minor nutrient elements to a level comparable to that of productive nursery soils (Table 4). The good growth of trees on substrata that would not yield even a dozen bushels of rye or oats per acre serves as noteworthy illustration of the mycotrophic potential and of the difference between the fertility concepts applicable to farm and forest soils.

TABLE 4. Effect of 32-year-old plantations of red pine on fertility of the surface 6-inch layer of geological matrix exposed by a removal of surface soil. Average results of 3 plantations (After Wilde and Iyer, 1962).

Horizons	Reaction pH	Total N%	Exchangeable p.p.m. Ca	Mg	Available p.p.m. P	K	B	Mn	Zn	Cu	Mo
Undisturbed original soil, 6" layer	4.8	0.230	530	64	36	64	0.81	16.2	7.3	3.8	0.77
Substratum 36-42"	5.2	0.003	40	9	56	9	0.45	2.2	1.1	0.3	0.16
Reforested substratum, 6" surface	4.9	0.082	114	11	44	27	0.73	5.7	3.2	2.5	0.29

Mycorrhizae and Biocides

The results of Wisconsin studies of the influence of eradicants on mycorrhiza-forming fungi varied considerably depending on the nature and concentration of chemicals. This essay includes the fraction of obtained information that appears to have lasting interest.

The effect of biocides on morphology and anatomy of mycorrhizal short roots was first studied in greenhouse cultures. The growing medium consisted of Plainfield outwash sand enriched in silicate minerals and harboring mycorrhiza fungi. This soil was treated with different amounts of chlordane, thiosan, benzene hexachloride, calomel, aluminum sulfate, allyl alcohol, formaldehyde, aldrin, and Stoddard solvent. *Pinus resinosa* and *Pinus radiata* were used as test plants.

The study of these eradicants included applications made at 2 and 3 times the recommended dosages. This was done to simulate a concentration found sporadically in nursery soils due to poor distribution of the chemicals and their illuvial accumulation. The heavy applications often depressed growth of seedlings (Fig. 10), caused partial destruction of the fungal mantle or cortical cells, restricted penetration of hyphae, and a reduced develop-

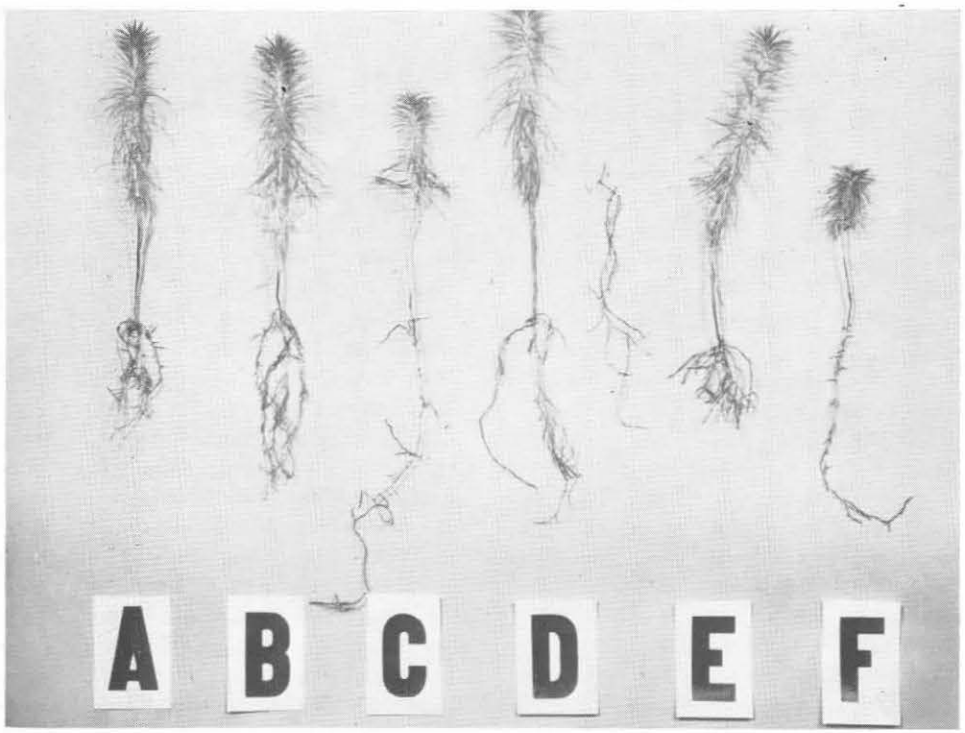

Figure 10. Growth of 1-year-old Pinus radiata *seedlings in a coarse sandy soil treated with different biocides at the indicated rates of application: A - Untreated soil; B - Chlordane, 10 lbs/A; C - Thiosan, 125 lbs/A; D - Allyl alcohol, 50 gal/A; E Benzene* hexachloride (gamma isomer), 1.0 lb/A; F - Calomel, 40 lbs/A.

ment of the Hartig net. Some biocides, especially benzene hexachloride and calomel, inhibited development of short roots, depressed respiration of root tips, and even injured non-mycorrhizal tissues of roots (Simkover and Shenefelt, 1952; Voigt, 1953; Persidsky and Wilde, 1955, 1960; Wilde and Persidsky, 1956; Wallis, 1957).

At certain concentrations, eradicants produced striking and specific modifications in the size and shape of mycorrhizal short roots (Fig. 11). These abnormalities often indicated a dangerous accumulation of the chemicals in nursery soils (Wilde and Persidsky, 1956).

Occasional absence of short roots of nursery stock suggested a possibility of a complete extermination of mycorrhiza-forming fungi and induced extensive greenhouse trials. These trials employed Carrington silt loam, a prairie soil lacking mycorrhiza formers, as a test medium. This soil was mixed with 0.2% by volume of soils collected from roots of non-mycorrhizal seedlings in the state nurseries located at Boscobel, Hayward, Rhinelander, and Wisconsin Rapids. Most of these soils had previously received repeated applications of thiosan, oil solvent, and chlordane or aldrin. The mixture of prairie and nursery soils was placed in glazed jars and planted with surface-sterilized seed of *Pinus resinosa* and *P. radiata*. With very few exceptions, the normal rapid growth of seedlings and gradual development of short roots manifested the presence of symbiotic fungi. Thus, these trials indicated that the entire annihilation of mycorrhiza-formers by these particular eradicants was precluded by either high resistance of fungi to the chemicals, or by the survival of symbionts in soil foci which remained free from critically concentrated biocides.

Concurrently with these trials, a detailed investigation of the influence of biocides on the performance of field planted nursery stock was conducted by G. K. Voigt. Seedlings of *Pinus banksiana*, *Pinus resinosa*, and *Picea glauca* were raised in the Griffith, Hugo Sauer, and Consolidated Industrial nurseries on soils treated with chlordane, thiosan, and Stoddard solvent. After 2 years of growth in the nurseries, the seedlings from treated and control plots were transplanted to cut-over lands in Adams and Oneida Counties. The last record, taken 10 years after field planting, has failed to disclose either a growth depressing or mycorrhiza deteriorating effect of eradicants (Voigt and Wilde, 1963). However, as was shown by experiences of recent years, the results obtained in these trials are not applicable to many newer biocides.

The introduction of methyl bromide and other fumigants has significantly changed the effects of chemical eradication on nursery stock, its development, fungal symbionts, and, most likely, potential performance under field conditions. Instead of a depressing effect, many recently introduced hydrocarbons produce a highly harmful stimulation of the growth of seedlings which disrupts their morphological balance and imparts many other undesirable properties (Iyer, 1968). Some of these chemicals, e.g., Vapam and Mylone, caused severe reduction or near annihilation of mycorrhizal short roots in nursery seedlings of *Pinus resinosa* and *Picea glauca* (Iyer, 1963; Iyer and Wilde, 1965). A fortunate exception among the new hydrocarbons is Dacthal, a herbicide which promoted the growth of seedlings without ill effect on the top-root ratio and mycorrhiza-formers (Iyer, 1965).

In some instances, either heavy or repeated applications of Vapam and other fumigants have drastically

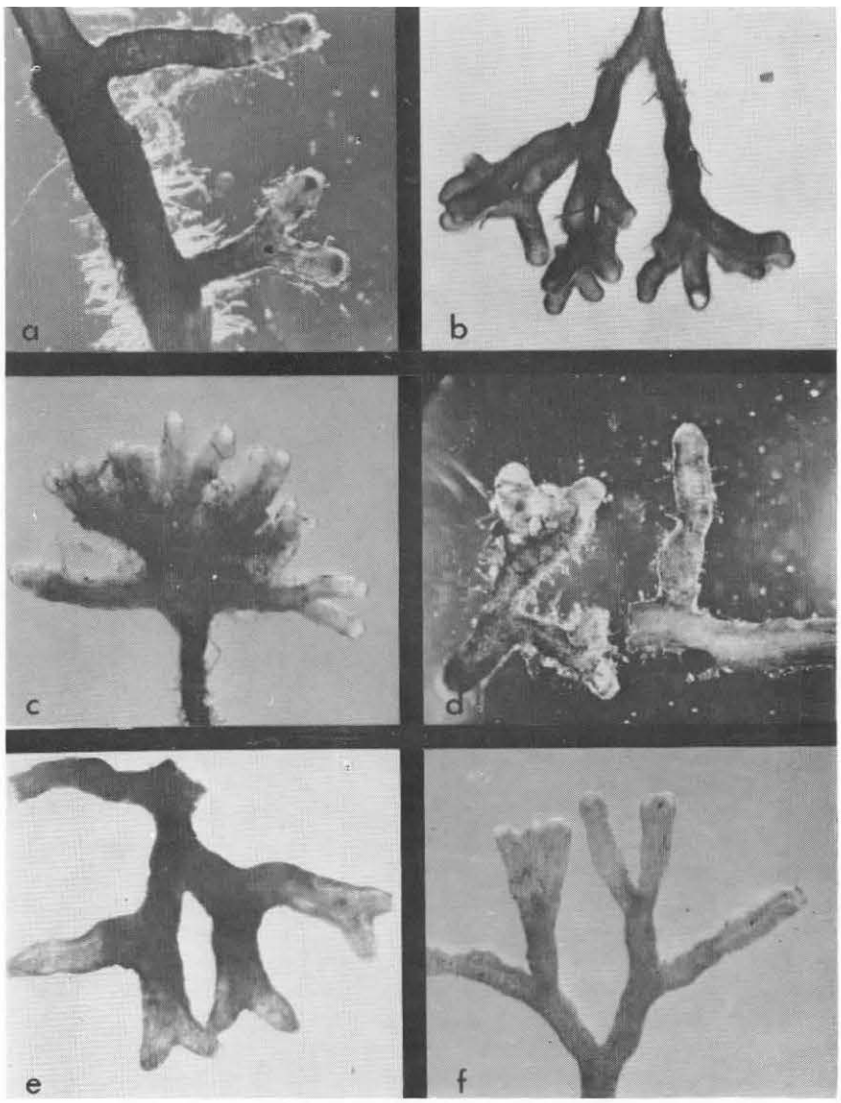

Figure 11. Morphology of mycorrhizal short roots of Pinus radiata *developed under the influence of various biocides - (a) Untreated sandy soil; normal bifurcate and simple short roots - (b) Formaldehyde at 2,600 gallons/A; coralloid clusters with greatly elongated peduncles - (c) Stoddard solvent, 50 gallons/A; clusters of coralloid pattern - (d) Vapam, 20 lbs/A; partly deteriorated bifurcate and simple short roots - (e) Chlordane, 5 lbs/A; elongated short roots of irregular slingshot pattern - (f) Benzene hexachloride, 1 lb/A; clovate or osteoform short roots serving as a very dependable identification of this particular chemical.*

changed the effect of the treatment by completely disrupting the mycotrophic mechanism of nursery soils and precluding uptake of even available nutrient elements (Henderson, and Stone, 1967; Lipas, 1968).

The most intriguing feature observed in our trials, is the ability of allyl alcohol to impart a delayed or transmitted high growth potential to tree seedlings (Wilde *et al.*, 1956).

In some greenhouse trials, designed to evaluate the effect of eradicants on the survival of mycorrhiza-forming fungi, *Pinus radiata* seedlings were first raised in biocide-treated soils harboring mycorrhiza-formers. At the end of 8 weeks the seedlings were transplanted into prairie soils free from mycorrhiza-formers. The subsequent growth of transplants then indicated the degree to which the biocides suppressed the symbiotic organisms.

When this technique was used in a study of the biocidic effects of allyl alcohol, totally unexpected and inexplicable results were observed.

A coarse sandy soil, autochthonously inoculated with *Cenococcum graniforme*, was collected from a mature stand of jack pine, *P. banksiana*, and used as the growing medium in greenhouse trials. This soil was placed in half-gallon glazed jars and treated with various biocides, including allyl alcohol; the latter was applied at the rate of 50 gallons per acre. Two weeks later, on December 4, the soils were seeded to Monterey pine, using 15 seeds per jar. All treatments were made in quadruplicate. The seedlings showed only small differences in growth at the end of 6 months.

Early in June, 4 average seedlings from each jar were transplanted into 2 gallon containers filled with Carrington prairie silt loam. After 8 months, none of the transplants, except those that were first raised in soils treated with allyl alcohol, exhibited differences in growth (Table 5). The transplants in alcohol-treated soil attained an abnormally large size and highly succulent structure (Fig. 12).

At the time of this study, the authors attributed the effect of allyl alcohol to "certain rhizospheric organisms, not identical with mycorrhiza-forming fungi." In the light of more recent studies (Yatazawa *et al.*, 1960), it is justifiable to believe that the drastic change in growth conditions was due to introduction into the prairie soil of *Trichoderma viride*. While this fungus does not form a union with internal tissues of roots, it converts into available form nutrients locked up in humates of prairie soils. This relationship provided probably one of the most striking examples of a rhizospheric symbiosis.

TABLE 5. Transmitted effect of allyl alcohol on the growth of Monterey pine seedlings. Average results on oven-dry basis (After Wilde *et al.*, 1956).

Treatment of sandy soil harboring mycorrhiza-forming fungi	Control	Thiosan 120 lbs/a	Chlordane 10 lbs/a	Allyl alcohol 50 gal/a
Weight of seedling after 6 months growth in sand cultures, g.	0.15	0.14	0.17	0.15
Weight of transplant after 8 months growth in prairie soil, g.	1.32	1.18	1.23	9.11

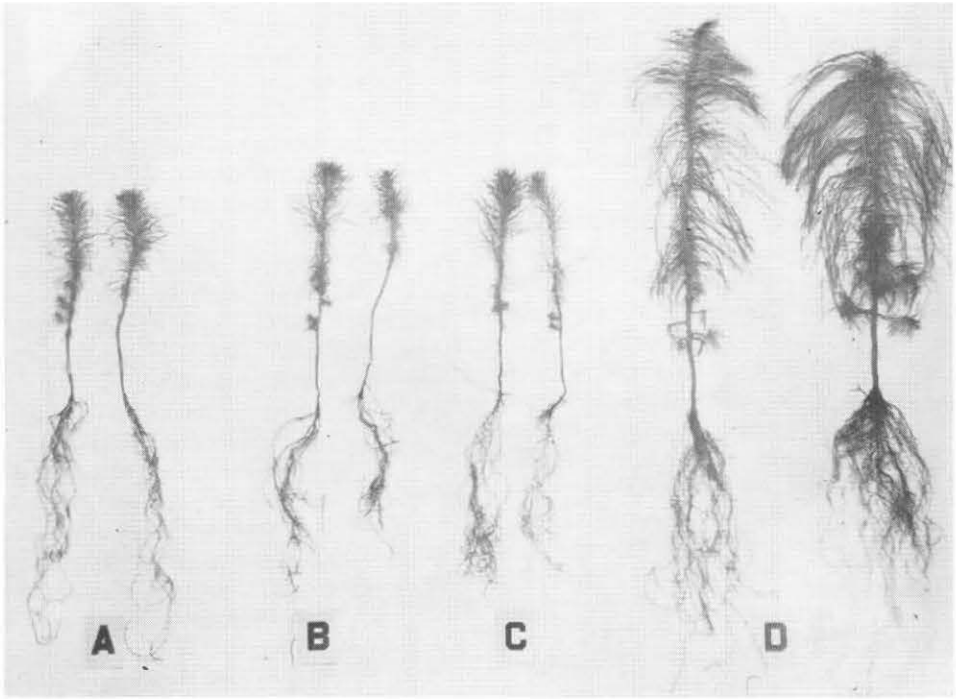

Figure 12. The growth of 8-month-old Monterey pine transplants in Carrington silt loam prairie soil. Prior to transplanting, the seedlings were raised for 6 months in mycorrhiza-inoculated sandy soil either untreated (A), or treated as follows: (B) thiosan, 120 lbs/A; (C) chlordane, 10 lbs/A; (D) allyl alcohol, 50 gallons/A.

Mycorrhiza and Silviculture

Many reports dealing with mycotrophy stress the importance of tree-fungus relationship in practical forestry. No one could deny the value of knowledge pertinent to symbiosis of higher and lower plants. However, speaking specifically of forestry practice, the importance of mycorrhizae is limited to rather rare cases in which attempts are made to afforest grassland soils by direct seeding or to produce nursery stock on such soils.

Following the suggestions of contemporary literature, attempts were made to render prairie soils suitable for the growth of trees from seed by the use of pure cultures of mycorrhiza-forming fungi. Greenhouse trials with subirrigated prairie soils were established by R. O. Rosendahl, who used *Pinus resinosa* and 7 species of mycorrhizal fungi, obtained from the Bureau of Plant Industry, USDA. These trials were initiated in the fall and failed to show any improvement in the growth of seedlings until late spring of the following year. At that time, plants in 4 jars inoculated with *Boletus felleus* began to show a rapid increase in growth, and in time exceeded by several hundred per cent all other plants in the production of dry matter. This

experiment was carried to its completion by field planting all greenhouse stock to virgin prairie soils in the vicinity of DeForest, Dane County. Subsequent growth of seedlings demonstrated the success and the vital importance of inoculation.

In spite of this promising achievement, it was decided to discontinue work on pure culture inoculations for the following 2 reasons: (1) experience even in controlled environments has shown the great uncertainty of a successful inoculation; (2) inoculation of prairie soils proposed to nursery stock production with pure cultures could not compete in speed and simplicity with less laborious introductions of symbionts. The author participated in inoculation of soils of the U.S. Licking Nursery in Missouri, the State Boscobel Nursery in Wisconsin, and several graded areas in other nurseries. In all cases, mycorrhizal fungi were permanently introduced by either broadcast applications of forest leafmold, or by transplanting seedlings produced on soils harboring symbionts. Successful inoculation was achieved in small scale trials by spraying the seed or roots of hydroponic stock with a suspension prepared from leafmold, or by hilling seedlings in forest soils.

During the past 2 decades many inoculations of grassland soils (so-called "mycorrhization" of soils) were conducted by the Russians in connection with establishment of shelterbelts by a direct seeding. This method of afforestation, however, is highly questionable. Most prairie soils present serious climatic and biotic obstacles to direct seeding: drying and freezing of the surface soil, competition of grass, destruction of seed by rodents, and damage by damping-off fungi. Therefore, planting mycorrhizal nursery stock is usually a more certain and economic way toward afforestation (Wilde, 1954).

Much has been written about the importance of introduction of specific mycorrhiza-forming fungi in connection with acclimatization of exotic tree species. This is particularly true of naturalization of *Pinus radiata* in New Zealand. In some statements, the results obtained with plantations of Monterey pine, *Pinus radiata*, were presented with unjustifiable implication that establishment of this tree required an introduction of specific mycorrhizal fungi from the United States. A soil sample collected in the autochthonous stand of *Nothofagus* of New Zealand successfully inoculated the prairie Carrington silt loam of Wisconsin and produced vigorous *Pinus radiata* and *Pinus resinosa* with abundant ectotrophic mycorrhizae.

The inference of Pachlewski (1953) as to the need for a replacement of "symbiotic fungi destroyed by soil cultivation in the nursery" shows the writer's little knowledge of nursery soils and of the enormous survival potential of mycorrhiza-formers. Even severe burning of slash and raw humus failed to delay inoculation of pine seedlings with mycorrhizae (Mikola *et al.*, 1964). Thus far, complete elimination of tree symbionts from forest soils was recorded only by a prolonged impoundment of water, such as occurs in beaver flowages (Wilde *et al.*, 1950).

Artificial introduction of mycorrhizal fungi into nursery soils subjected to application of biocides, suggested by Hacskaylo (1961), is likely to be practicable only in the form of leafmold application. Pure cultures of fungi have little chance to withstand toxicity of soil treated with eradicants, or competition of indigenous fungi (Laiho and Mikola, 1964; Mikola, 1965.)

Summary

Investigations conducted during the past quarter century by Wisconsin students of mycotrophy reemphasize the essentiality of symbiotic fungi in nutrition of trees and other woody plants. Trials employing symbiont-free prairie soils and hydroponic cultures definitely disproved the existence of "autotrophic" or mycorrhiza-free trees. Fungi forming mycorrhizae with all investigated trees were invariably obtained not only from Wisconsin forest soils, but also from soils of cold (Alaska) and tropical (India) regions. This was true of both forested and cutover soils, including those of extremely unfavorable composition, such as windblown sands, moss peats, layers resting on permafrost, and ferralized clays. The constant presence of mycorrhiza-forming fungi in deforested soils that were for many decades utilized by farming gave rise to the credo "Once mycorrhizal soil, forever mycorrhizal soil."

On the other hand, Wisconsin studies have failed to detect mycorrhiza-forming fungi in prairie soils of both fine and coarse texture, and our findings are in discord with the claims of some Russian investigators. In fact, our research suggested that the absence of tree symbionts is largely responsible for the very persistence of the intra-zonal prairies in the humid climate of the American Midwest. In prairie soils of Wisconsin supporting windbreaks, the distribution of mycorrhiza-formers is strictly delineated by the extension of tree roots. Introduction of mycorrhiza-formers into prairie soils has never failed to produce increases as much as several hundred per cent in the growth of both coniferous and deciduous tree seedlings. These trials demonstrated in a most dramatic manner the role which microorganisms occasionally play in the life of higher plants.

The results of Wisconsin investigations of mycotrophy are in agreement with the mineral theory of Stahl. The effect of symbiotic fungi on the release of nutrients available to trees from raw or inert organic matter and unweathered silicate minerals was repeatedly demonstrated. As disclosed by field and greenhouse studies, the conversion of immobilized nutrients is accomplished partly by mycelia imbedded in the cortex of roots, and partly or entirely by extra-matrical, juxtaposed mycelia. The latter form around the roots either lanose mantles, the *mycochlamydes*, or grumose mycelia-penetrated organo-mineral sheaths, the *mycoplasts*.

The peripheral mycelia derive their energy from decomposition of root sloughings and enrich the rhizosphere in chelating agents that liberate available nutrients from undecomposed organic matter and unweathered minerals. Chelating agents are largely responsible for the ability of trees to produce high yields on soils whose analyses show a deficiency of available nutrients. The essentiality of epirhizal mycelia is expressed by the maxim that a tree or other lignophyte removed from the soil is only a part of the whole plant, a part surgically separated from its rhizospheric digestive organ.

Observations of greenhouse and nursery grown tree seedlings suggest that in symbiotrophy the fungus fulfills the requirements specified by the carrier concept of the uptake of nutrients, especially phosphorus.

Observation of tree roots in dense forest stands indicated that mycorrhizal short roots present only one (and

not the most important) feature of the symbiotrophic mechanism. An abundance of these appendages is an incidental characteristic largely common to seedlings of greenhouse cultures, nursery beds, and young plantations, *i.e.*, plants exposed to full sunlight and producing excess of carbohydrates.

Regardless of the nature of introduced inoculum, the growth of tree seedlings was invariably correlated with fertility of inoculated soil, thus indicating that fungal symbionts act entirely as quasi-catalytic agents.

Application of some biocide fumigants to nursery soils reduced, or temporarily eliminated, mycorrhiza-forming fungi and thereby arrested the uptake of phosphorus, even though an abundant supply of this element was present in the soil in available form.

The introduction of fungal symbionts into prairie soils assigned to nursery stock production was successfully accomplished by broadcast and rototilling into the soil the surface layers of forest soils (0.1 to 0.2% by volume), or by transplanting on the area mycorrhizal seedlings.

N e i t h e r deforestation, prolonged farming use of the land, nor forest fires caused a complete annihilation of mycorrhiza-formers. The absence of these organisms in once-forested soils was detected only in submerged layers of peat and in soils of abandoned beaver flowages that remained for many years under impounded water.

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